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Nicotine Induces a Long QT Phenotype in Kcnq1-Deficient Mouse Hearts

TOSHIMASA TOSAKA, MATHEW C. CASIMIRO, QI RONG, SRIHARI TELLA, MICHELLE OH, ALEXANDER N. KATCHMAN, JOHN C. PEZZULLO, KARL PFEIFER, and STEVEN N. EBERT

Department of Pharmacology, Georgetown University Medical Center, Washington, DC (T.T., Q.R., M.O., A.N.K., J.C.P., S.N.E.); Laboratory of Mammalian Genes and Development, National Institute of Child Health and Human Development/National Institutes of Health, Bethesda, Maryland (M.C.C., K.P.); Drug and Chemical Evaluation Section, Office of Diversion Control, Drug Enforcement Administration, Washington DC (S.T.)

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ABSTRACT

We have previously shown that targeted disruption of the mouse *Kcnq1* gene produces a long QT phenotype in vivo that requires extracardiac factors for manifestation (Casimiro et al., 2001). In the present study, we explore the hypothesis that autonomic neuroeffector transmission represents the "extracardiac" stimulus that induces a long QT phenotype in mouse hearts lacking Kcnq1. Using the isolated perfused (Langendorff) mouse heart preparation, we challenged wild-type (*Kcnq1*$^{+/+}$) and mutant (*Kcnq1*$^{-/-}$) mouse hearts with nicotine, an autonomic stimulant. ECGs were recorded continuously, and QT intervals were compared at baseline and peak nicotine-induced heart rates. No significant differences in QT or any other ECG parameters were observed in *Kcnq1*$^{+/+}$ versus *Kcnq1*$^{-/-}$ hearts at baseline. In the presence of nicotine, however, the JT, QT, and rate-corrected QT (QTc) intervals were significantly prolonged in *Kcnq1*$^{-/-}$ hearts relative to *Kcnq1*$^{+/+}$ hearts (e.g., QTc = 92 ± 11 ms versus 66 ± 2 ms, respectively, $p < 0.01$). Similar findings were obtained when the hearts were challenged with either epinephrine or isoproterenol (0.1 μM each), thereby suggesting that sympathetic stimulation drives the long QT phenotype in Kcnq1-deficient hearts. This idea is supported by in vivo ECG data obtained from unrestrained conscious mice using radiotelemetry recording techniques. Again, no significant ECG differences were observed in *Kcnq1*$^{-/-}$ versus *Kcnq1*$^{+/+}$ mice at baseline, but handling/injection stress led to significant QTc increases in *Kcnq1*$^{-/-}$ mice relative to wild-type controls (11 ± 3 versus −1 ± 1%, respectively, $p < 0.05$). These data suggest that sympathetic stimulation induces a long QT phenotype in Kcnq1-deficient mouse hearts.

Long QT syndrome (LQTS) is a disorder of impaired cardiac repolarization, manifesting long QT intervals on ECG recordings and resulting in increased risk of potentially lethal ventricular arrhythmias known as "Torsades de Pointes" (TdP) (Viskin, 1999; Camm et al., 2000;Chiang and Roden, 2000). LQTS can be acquired or inherited. Both forms ultimately appear to influence ion channel activity in the heart (Roden and Spooner, 1999). Patients with the most prevalent form of congenital LQTS, *LQT1*, have mutations or deletions in the *KCNQ1* gene (previously known as *KvLQT1*), which encodes for a cardiac potassium channel thought to play an important role in cardiac repolarization (Wang et al., 1996; Yang et al., 1997). LQT1 patients are at high risk for developing TdP arrhythmias particularly during periods of acute stress when sympathetic tone and β-adrenergic stimulation are high (Ackerman et al., 1999; Schwartz et al., 2001).

We have recently described the initial characterization of a mouse model for Jervell and Lange-Nielsen syndrome, a relatively rare form of LQT1 that is also associated with severe bilateral deafness (Casimiro et al., 2001). This model was created through targeted disruption of the endogenous mouse *Kcnq1* gene. We measured ECGs in sedated mice and demonstrated that *Kcnq1*-deficient (*Kcnq1*$^{-/-}$) mice have impaired cardiac repolarization, as indicated by extended rate-corrected QT (QTc) intervals and altered T-wave morphologies compared with those found in wild-type littermates. In contrast, we found that these "baseline" ECG differences observed between diazepam-sedated *Kcnq1*$^{+/+}$ and *Kcnq1*$^{-/-}$ mice in vivo were not evident in ECG recordings from isolated perfused (Langendorff) mouse heart preparations. One specific hypothesis that could account for this apparent discrepancy is that autonomic regulation of Kcnq1

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ABBREVIATIONS. LQTS, long QT syndrome; TdP, Torsades de Pointes; QTc, rate-corrected QT interval; VPC, ventricular premature contraction; NSVT, nonsustained ventricular tachycardia.

function in the heart is needed to produce the observed cardiac repolarization defects in *Kcnq1*$^{-/-}$ mice. In the present study, we directly test this hypothesis by evaluating ECG responses to autonomic stimulants in *Kcnq1*$^{-/-}$ and *Kcnq1*$^{+/+}$ hearts.

Nicotine is a classical autonomic neuroeffector (De Biasi, 2002). In isolated perfused heart preparations, administration of nicotine produces a brief modest slowing of heart rate that is immediately followed by a much larger transient increase in heart rate (Kottegoda, 1953; Ji et al., 2002). We and others have shown that the nicotine-induced decrease in heart rate is likely a parasympathetic response because it could be selectively blocked by the muscarinic antagonist atropine (Kottegoda, 1953; Ji et al., 2002). Thus, nicotine probably facilitates local release of acetylcholine that, in turn, acts on muscarinic receptors as part of a parasympathetic response. In contrast, the nicotine-induced increase in heart rate is thought to occur via facilitation of norepinephrine release from sympathetic nerve terminals and/or intrinsic cardiac adrenergic cells (Burn and Rand, 1958; Westfall and Brasted, 1972, 1974; Ji et al., 2002). For example, Westfall and Brasted (1972, 1974) showed that increased amounts of [^{3}H]norepinephrine were found in perfusates from preloaded guinea pig hearts following nicotine administration. Kruger et al. (1995) came to similar conclusions by directly measuring norepinephrine content using high-pressure liquid chromatography with electrochemical detection of perfusates from isolated perfused guinea pig hearts and human atrium. Furthermore, pretreatment with the catecholamine-depleting agent reserpine or the β-adrenergic antagonist timolol blocked nicotine-induced increases in heart rate (Burn and Rand, 1958; Ji et al., 2002).

In the present study, we show that nicotine causes prolongation of the QT interval in *Kcnq1*$^{-/-}$ hearts. Similar QT-lengthening effects were also observed in these hearts when perfused with epinephrine or isoproterenol, suggesting that sympathetic stimulation can produce a long QT phenotype in *Kcnq1*$^{-/-}$ mouse hearts. In vivo data recorded via radiotelemetry from surgically implanted ECG electrodes further support a role for a sympathetic mechanism since significant QTc increases were observed in *Kcqn1*$^{-/-}$ but not *Kcnq1*$^{+/+}$ mice following periods of acute stress.

Materials and Methods

Drugs and Chemicals. All drugs and chemicals were purchased from the Sigma-Aldrich (St. Louis, MO). Nicotine, epinephrine, and isoproterenol were prepared as 100 mM stock solutions and stored in small aliquots at −80°C. Immediately before use, the drug(s) were thawed and diluted in either vehicle (0.9% saline and 0.1% ascorbic acid) for injection or in Tyrode's solution for Langendorff perfusion.

Animals. Kcnq1$^{-/-}$ and *Kcnq1*$^{+/+}$ mice were generated and housed as previously described (Casimiro et al., 2001). All experiments were conducted in strict concordance with the guidelines provided by the Georgetown University Animal Care and Use Committee and the National Institutes of Health.

Isolated Perfused Heart Experiments. The isolation and perfusion of the adult mouse heart was performed essentially as described (Ji et al., 2002). Briefly, the hearts were equilibrated by retrograde aortic perfusion using a constant flow rate of 2 ml/min with freshly prepared, 37°C, oxygenated Tyrode's solution for 20 to 30 min before the addition of nicotine (100 μM directly to the perfusion buffer). Pilot experiments using different concentrations of nicotine indicated that 100 μM nicotine produced a maximal heart rate increase, similar to what we previously observed with rat hearts (Ji et al., 2002). The heart was perfused with the nicotine solution for 5-min and then for 20 to 30 min with drug-free Tyrode's buffer ("washout" period). The hearts were then challenged with either epinephrine (0.1 μM) or isoproterenol (0.1 μM) in the perfusion buffer. ECGs were recorded continuously from electrodes placed around the heart in simulated "Einthoven" fashion. The data were analyzed using customized LabView 5.1 data analysis software (National Instruments Corp., Austin, TX), as previously described. ECG signals were averaged over 30-s intervals before analysis to reduce "noise" interference. Measurement of ECG parameters was manually performed by at least two different investigators, both of whom were blinded to the genotypes, using criteria that have been previously established (Casimiro et al., 2001).

Surgical Implantation of ECG Radiotransmitters. Three *Kcnq1*$^{-/-}$ and three *Kcnq1*$^{+/+}$ male littermates 8 to 12 weeks old and weighing approximately 30 g were anesthetized with isoflurane (1–1.5%). The scapula, thorax, and abdominal regions were shaved and cleaned with iodine and alcohol solutions. An incision large enough to accommodate the implant was made in the middle of abdomen along the axis of the body immediately cranial to the sternum. A pouch was made under the skin by separating the skin from the underlying tissue using a blunt instrument, and an ECG transmitter (Data Sciences International, St. Paul, MN) was inserted into the pouch. The negative lead was tunneled under the skin to right shoulder position, and the positive lead was tunneled under the skin to the lowest left rib. A plastic sleeve was placed over the exposed tip of the wire lead, and the lead wires were fastened to the tissue. All incisions were closed by suturing. Injections of enrofloxacin (3 mg/kg i.m.) and Buprenex (0.1 mg/kg i.p.) were administered twice daily for the first 2 to 3 days after the surgeries to safeguard against infection and to alleviate postoperative pain, respectively. The mice were allowed to recover for at least 1 week before experimentation.

Radiotelemetry ECG Recordings and Analysis. ECG data were recorded from unrestrained mice using radiotelemetry, as described (Tella et al., 1999). Briefly, mice in their home cages were placed on the top of the transmitter receivers, which in turn were placed in sound-attenuated chambers. The transmitter data input to the receivers was transferred to a computer via a matrix box (Data Sciences International). Data collection was performed using Dataquest software, and off-line data analyses were performed using Physiostat ECG analysis software, version 3.1 (Data Sciences International). QT intervals were measured manually from printouts of the ECG data using established methods (Casimiro et al., 2001). QTc values were derived using the following formula: QTc = QT/SQRT(RR/100) (Mitchell et al., 1998).

Statistical Analyses. Unless otherwise specified, data are reported as the mean ± S.D. Statistical significance was determined by the use of the Student's t test, with $p < 0.05$ required to reject a null hypothesis. QT versus RR data were analyzed by linear least-squares regression, and 95% confidence bands were placed around the fitted line. Correlations were tested for significance by the Student's t transformation. The difference between the correlations was tested by the Fisher z transformation, and the difference between the two slopes was tested by analysis of variance.

Results

Isolated Perfused (Langendorff) Mouse Heart Experiments. To determine whether autonomic stimulation could influence cardiac repolarization in Kcnq1-deficient mouse hearts, we evaluated ECGs from spontaneously-beating, isolated, perfused *Kcnq1*$^{+/+}$ and *Kcnq1*$^{-/-}$ hearts immediately before and after nicotine (100 μM) administration. An example of the heart rate changes associated with the drug challenges employed for these experiments is depicted in Fig. 1.

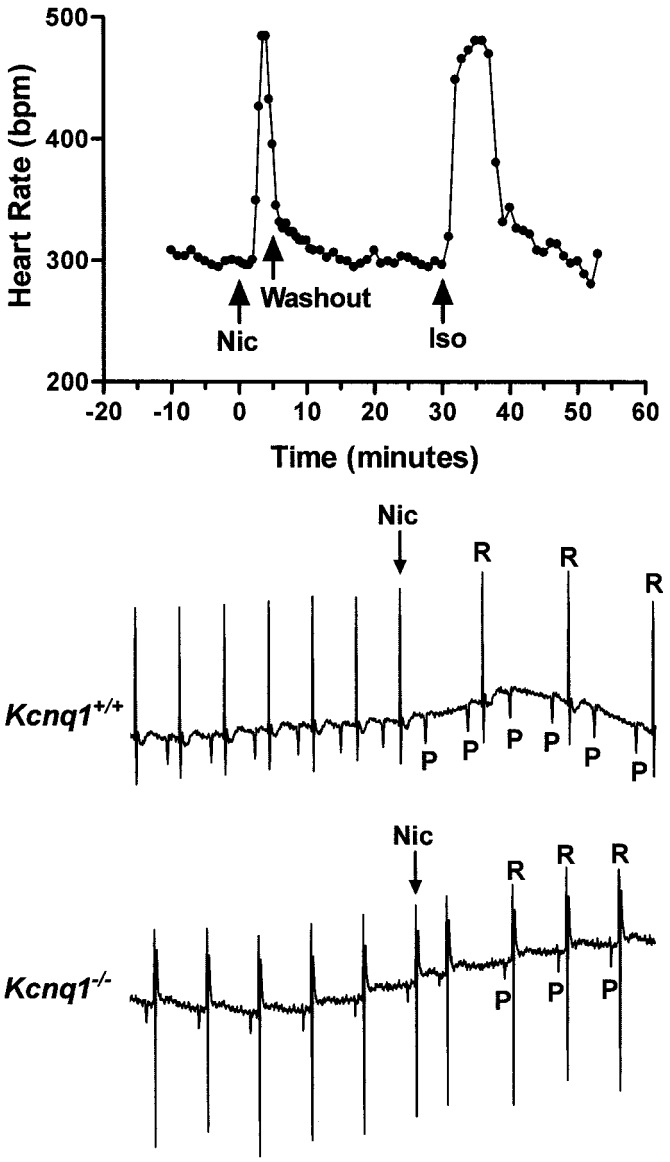

Fig. 1. Example of heart rate changes induced by nicotine and isoproterenol in isolated perfused mouse hearts. Upper panel, the heart used in the example shown was from an adult *Kcnq1*$^{-/-}$ mouse. Following a 20- to 30-min stabilization period of perfusion in the absence of drugs, nicotine (100 μM) was added to the perfusion buffer at the "zero" time-point (Nic, arrow). Accounting for the time it takes for the perfusion buffer to travel from the reservoir beaker to the heart, there was a typical lag-period of ~2-min between the time nicotine was added to the perfusion buffer and the timepoint when the nicotine solution came into contact with the heart. After 5-min of perfusion with nicotine, the perfusion buffer was switched back to normal Tyrode's (i.e., no drugs), thereby beginning the "washout" period (arrow). Isoproterenol (0.1 μM) was then added to the perfusion buffer approximately 25 min later, as designated (Iso, arrow). Lower panel, examples of raw ECG recordings from *Kcnq1*$^{+/+}$ and *Kcnq1*$^{-/-}$ mouse hearts at the timepoint when nicotine came into contact with the heart (Nic, arrow; note: this occurred ~2 min after addition of nicotine to the perfusion buffer due to the lag-time needed for nicotine to travel from the perfusion buffer reservoir to the heart). A 2:1 atrioventricular conduction block was induced in the *Kcnq1*$^{+/+}$ heart, but not in the *Kcnq1*$^{-/-}$ heart. "P" and "R" indices are indicated as shown. The time-scale for both ECG traces shown was 2 s.

The baseline heart rate in these experiments was ~300 bpm, and nicotine elicited a transient increase up to ~400 to 500 bpm in both strains. Following the 5-min perfusion with nicotine, the hearts were perfused with drug-free Tyrode's buffer for an additional 20-min ("washout" period), during which time the heart rates returned to baseline values. A second sympathetic challenge was then directly administered by adding either the β-adrenergic receptor agonist isoproterenol (0.1 μM) or the adrenal stress hormone epinephrine (0.1 μM). This resulted in a second transient increase in heart rate.

The example shown in the top panel of Fig. 1 was measured from a *Kcnq1*$^{-/-}$ heart, but the overall heart rate responses to nicotine were similar to those found in *Kcnq1*$^{+/+}$ control hearts (compare RR values in Tables 1 and 2), with one exception. In *Kcnq1*$^{+/+}$ hearts, nicotine typically caused an initial brief slowing of the heart rate due to atrioventricular conduction block that recovered within a few seconds (observed in four of six *Kcnq1*$^{+/+}$ hearts). One such example is shown in the lower panel of Fig. 1. In contrast, none of the six *Kcnq1*$^{-/-}$ hearts tested showed any sign of nicotine-induced atrioventricular block, although minor rate changes such as that shown in Fig. 1 (bottom trace) were typically observed in *Kcnq1*$^{-/-}$ hearts at the start of nicotine treatment. Despite this difference, the subsequent rise in heart rate in response to nicotine was similar in *Kcnq1*$^{+/+}$ and *Kcnq1*$^{-/-}$ hearts.

To compare the effects of these sympathetic challenges in *Kcnq1*$^{+/+}$ and *Kcnq1*$^{-/-}$ hearts, various ECG parameters were measured at baseline and again at the peak drug-induced heart rate. Representative sample ECG recordings from *Kcnq1*$^{+/+}$ and *Kcnq1*$^{-/-}$ mouse hearts before and after nicotine treatment are displayed in Fig. 2. Before nicotine treatment (baseline), similar QT intervals were observed in *Kcnq1*$^{+/+}$ and *Kcnq1*$^{-/-}$ hearts (Fig. 2, A and C). Following the addition of nicotine, however, there was a clear difference in QT responses, with *Kcnq1*$^{-/-}$ hearts displaying substantially longer QT durations than the *Kcnq1*$^{+/+}$ hearts (Fig. 2, B and

TABLE 1
Comparison of baseline ECG parameters from *Kcnq1*$^{+/+}$ and *Kcnq1*$^{-/-}$ mouse hearts

ECG Parameters: Baseline	*Kcnq1*$^{+/+}$	*Kcnq1*$^{-/-}$	*p* Value
	$n = 6$		
RR (ms)	218 ± 35	198 ± 26	0.28
PR (ms)	36 ± 5	39 ± 5	0.32
QRS (ms)	9 ± 6	8 ± 2	0.71
QT (ms)	91 ± 15	94 ± 20	0.77
QTc (ms)	61 ± 7	64 ± 7	0.48
JT (ms)	76 ± 10	81 ± 16	0.53
T-Wave area (mV · ms × 10^{-1})	182 ± 62	223 ± 126	0.49

TABLE 2
Comparison of ECG parameters from nicotine-treated *Kcnq1*$^{+/+}$ and *Kcnq1*$^{-/-}$ mouse hearts

ECG Parameters: Nicotine (100 μM)	*Kcnq1*$^{+/+}$	*Kcnq1*$^{-/-}$	*p* Value
	$n = 4$[a]	$n = 5$[b]	
RR (ms)	142 ± 11	148 ± 12	0.47
PR (ms)	31 ± 6	40 ± 17	0.35
QRS (ms)	9 ± 7	12 ± 8	0.57
QT (ms)	79 ± 4	111 ± 11	<0.001
QTc (ms)	66 ± 2	92 ± 11	<0.01
JT (ms)	69 ± 5	102 ± 11	<0.001
T-Wave area (mV · ms × 10^{-1})	291 ± 111	530 ± 340	0.22

[a] No analysis was obtained for 2 of the 6 *Kcnq1*$^{+/+}$ hearts following nicotine treatment because of persistent atrioventricular block (recovered after several minutes).
[b] No analysis was obtained for 1 of the 6 *Kcnq1*$^{-/-}$ hearts following nicotine treatment due to development of ventricular tachycardia (recovered after ~1 min).

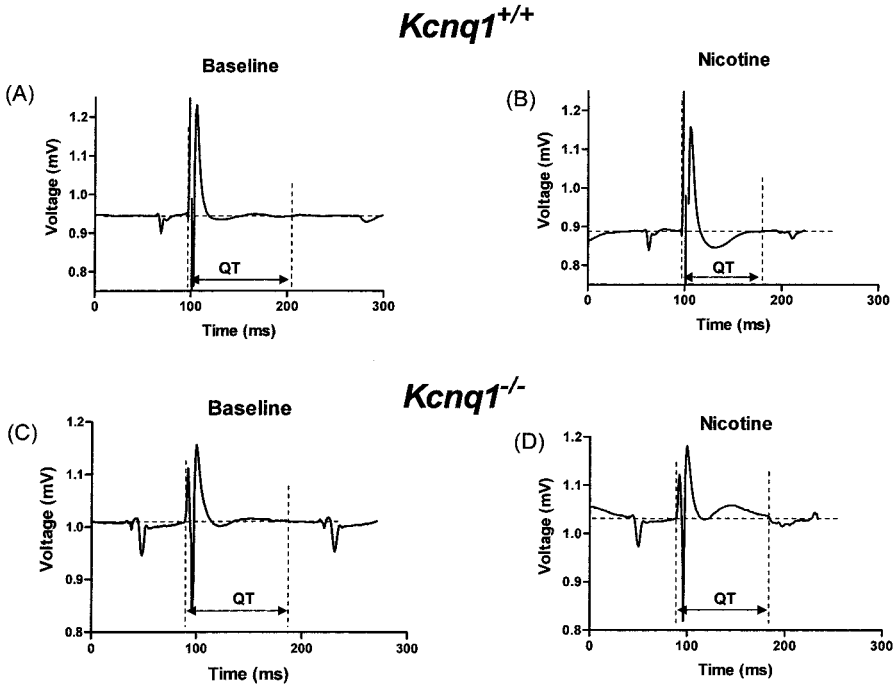

Fig. 2. Examples of ECG recordings from isolated perfused *Kcnq1*$^{+/+}$ (A and B) and *Kcnq*$^{-/-}$ (C and D) mouse hearts before (A and C) and after (B and D) nicotine (100 μM) administration. The ECG traces represent recordings that were signal-averaged over a 30-s interval immediately before the addition of nicotine (baseline) and again at the peak nicotine-induced heart rate (nicotine). To facilitate comparison of the traces, they were aligned vertically using the beginning of the QRS complex as a fixed reference point. The horizontal dashed line represents the isoelectric line for each trace, and the vertical dashed lines define the QT interval.

D). In *Kcnq1*$^{+/+}$ hearts, there was a tendency for the T-wave end to become "flat" upon return to the isoelectric line (dashed horizontal lines). In contrast, *Kcnq1*$^{-/-}$ hearts developed dramatic T-wave changes with a clearly distinct "end" during peak nicotine responses (compare Fig. 2D with A–C).

Quantitative assessment of these ECG data demonstrated that the QT, QTc, and JT intervals were significantly longer in the *Kcnq1*$^{-/-}$ hearts compared with the *Kcnq1*$^{+/+}$ hearts in the presence of nicotine (Tables 1 and 2). We also noted a trend toward increased PR intervals and T-wave areas in *Kcnq1*$^{-/-}$ mice following nicotine treatment, although these changes were not statistically significant. In all other respects, similar ECG parameters were observed in *Kcnq1*$^{+/+}$ and *Kcnq1*$^{-/-}$ mice.

To further evaluate the QT phenotype in these mouse hearts, we compared the QT-RR relationship for each strain by examining the degree of QT change over the range of RR values recorded during the nicotine challenge experiments. As shown in Fig. 3A, the QT interval shortens at faster heart rates (as the RR interval shortens) in *Kcnq1*$^{+/+}$ mouse hearts, producing a linear QT-RR relationship ($r^2 = 0.466$, $p < 0.001$). In contrast, there was no QT-RR correlation ($r^2 = 0.002$, $p = 0.72$) in *Kcnq1*$^{-/-}$ mouse hearts (Fig. 3B). These data indicate that, unlike *Kcnq1*$^{+/+}$ mouse hearts, the QT changes in *Kcnq1*$^{-/-}$ mouse hearts did not adjust to the sympathetically driven increases in heart rate.

To verify that the nicotine-induced ECG differences observed were primarily due to its sympathetic activity as opposed to the relatively minor parasympathetic effects that nicotine is known to exert in these preparations (Kottegoda, 1953; Ji et al., 2002), we also analyzed the QTc responses following epinephrine and isoproterenol administration. As shown in Fig. 4, these adrenergic agents stimulated similar heart rate increases in both strains. In contrast, the increase in QTc interval was significantly larger in the *Kcnq1*$^{-/-}$ hearts compared with *Kcnq1*$^{+/+}$ hearts ($p < 0.05$, n = 6/group; Fig. 4A). These effects were remarkably similar to

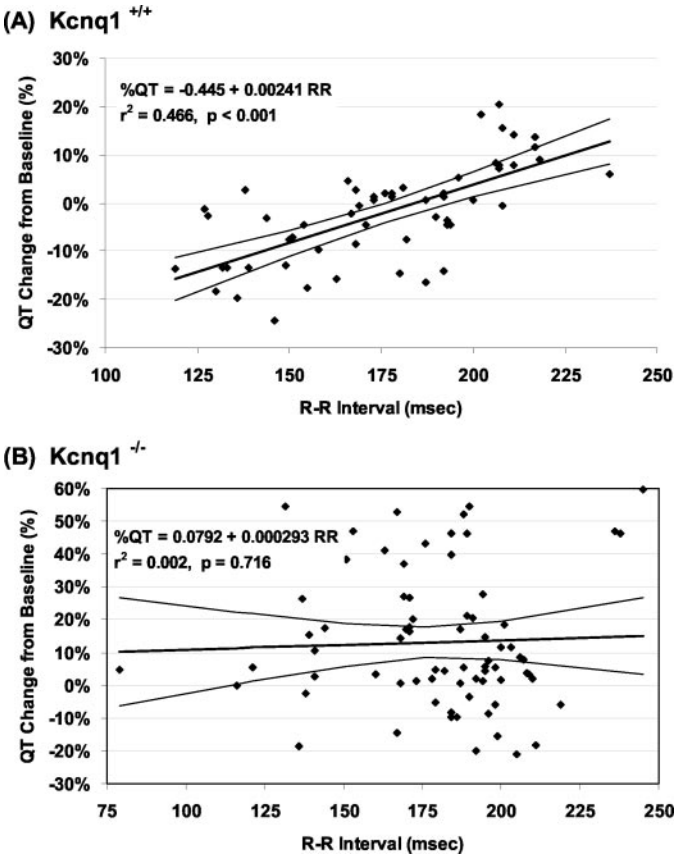

Fig. 3. QT-RR relationship in isolated perfused (A) *Kcnq1*$^{+/+}$ and (B) *Kcnq1*$^{-/-}$ mouse hearts in response to nicotine challenge. The QT values were normalized to baseline (average QT over the 5-min period immediately preceding administration of nicotine) for each mouse heart and expressed as the degree of QT change (%QT) from baseline for each RR value (measured from baseline to peak heart rate). The linear equations and correlation coefficients (R^2 values) are provided for each data set. Confidence limits (95%) are shown.

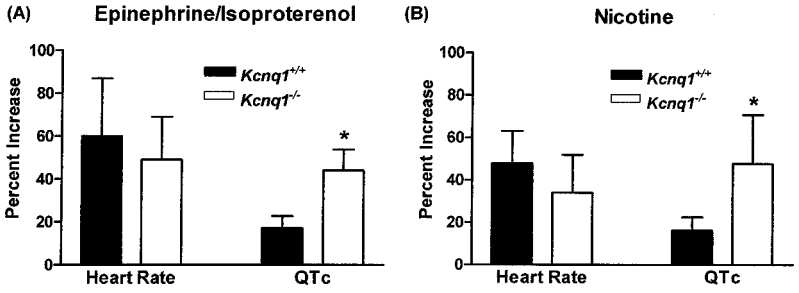

Fig. 4. Changes in heart rate and QTc induced by sympathomimetic drug stimulation in isolated perfused $Kcnq1^{+/+}$ and $Kcnq1^{-/-}$ mouse hearts. A, heart rate (derived from RR values) and QTc measurements were compared immediately before administration of either 0.1 μM epinephrine (n = 4/genotype) or isoproterenol (n = 2/genotype) and again at the peak drug-induced heart rate. Epinephrine and isoproterenol produced similar increases in heart rates, and the results of these treatments were combined to evaluate the overall effect of adrenergic stimulation on the degree of heart rate and repolarization (QTc) change that occurred in $Kcnq1^{+/+}$ and $Kcnq1^{-/-}$ hearts. B, heart rate and QTc changes induced by nicotine (100 μM).

those produced by nicotine (Fig. 4B), thereby suggesting that sympathetic stimulation induced the long QT phenotype in $Kcnq1^{-/-}$ hearts.

Radiotelemetry Experiments. To test the hypothesis that sympathetic stimulation provokes impaired cardiac responses in $Kcnq1^{-/-}$ mice in vivo, we attempted to record ECGs from surgically implanted radiotelemetry electrode transmitters in $Kcnq1^{-/-}$ and $Kcnq1^{+/+}$ mice during exercise-stress testing, such as swimming or running on a treadmill. These strategies proved to be ineffective because $Kcnq1^{-/-}$ mice could not perform either of these tests due to the balance problems associated with their inner ear defects (Kcnq1 is required for endolymph homeostasis/biosynthesis in both humans and mice). As an alternative strategy, we next recorded ECGs from mice following injection with nicotine, a sympathetic stimulant. Despite attempts to habituate the mice to handling, however, we found that the injection procedure itself consistently produced sympathetic activation, as reflected by the rapid transient increases in heart rate that occurred following vehicle (saline) injection. Therefore, we compared ECG recordings before and after saline injections to measure the effects of "injection stress" in wild-type and mutant mice.

$Kcnq1^{+/+}$ and $Kcnq1^{-/-}$ mice displayed similar heart rates at baseline and similar stress-induced rate increases (Fig. 5A). At peak heart rates, $Kcnq1^{-/-}$ mice typically had longer QT intervals, as shown in the example ECG traces depicted in Fig. 5B (see Fig. 5C for average QTc values). The QTc in the $Kcnq1^{-/-}$ group increased by an average of 11 $\pm$ 3% ($p < 0.05$) compared with -1 ± 1% QTc change in the $Kcnq1^{+/+}$ group (Fig. 5D). These results show that the absence of Kcnq1 expression can lead to a stress-induced Long QT phenotype in adult mice.

Arrhythmia Assessments. We did not observe any episodes of Torsades-like arrhythmias nor was there any indication of ventricular fibrillation in either mouse strain from any of the recording sessions. We did, however, observe occasional ventricular premature contractions (VPCs) and short runs of nonsustained ventricular tachycardia (NSVT) primarily in one $Kcnq1^{-/-}$ mouse during radiotelemetry recordings. These arrhythmia events occurred during baseline and after injection stress. The other $Kcnq1^{-/-}$ mice recorded by radiotelemetry showed little or no occurrence of arrhythmias, as was also found for the $Kcnq1^{+/+}$ mice. A similar situation was observed during the isolated perfused mouse heart experiments. For example, none of the $Kcnq1^{+/+}$ mouse hearts developed ventricular arrhythmias, but one of the $Kcnq1^{-/-}$ mouse hearts developed NSVT immediately following the administration of nicotine. Examples of some arrhythmia episodes recorded in vivo and in vitro are shown in Fig. 6.

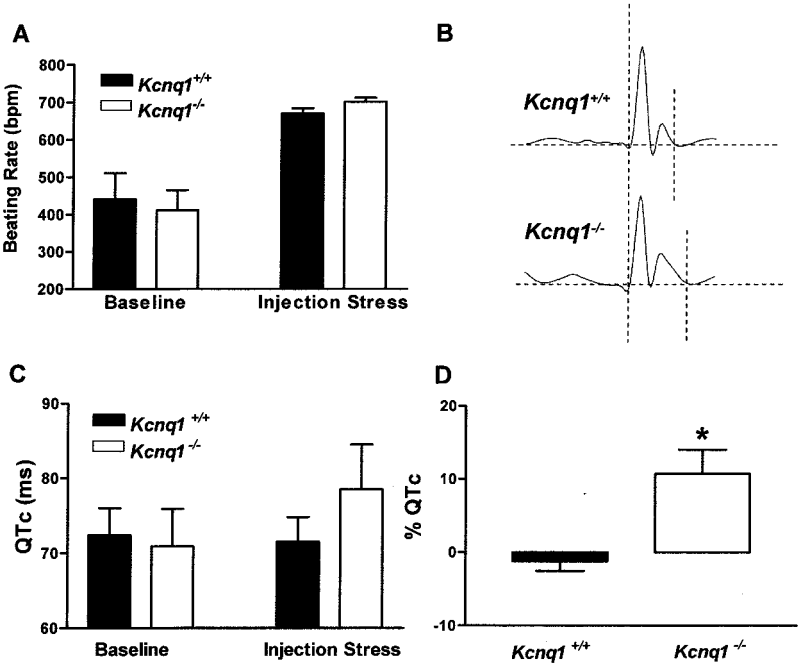

Fig. 5. Comparison of in vivo ECG data recorded via surgically implanted radiotelemetric transmitters in conscious, unrestrained $Kcnq1^{+/+}$ and $Kcnq1^{-/-}$ mice. A, comparison of baseline and peak stress-induced heart rates. B, examples of ECG traces recorded at peak stress-induced heart rates. The traces were signal-averaged over 10-s intervals. The RR intervals were similar (~84 ms, corresponding to a heart rate of ~714 bpm), but the QT interval was substantially longer in the $Kcnq1^{-/-}$ mouse (demarcated by the vertical dashed lines). C, QTc measurements from $Kcnq1^{+/+}$ (filled bars) and $Kcnq1^{-/-}$ (open bars) mice before and after injection stress. D, degree of QTc change (%QTc) induced by injection stress.

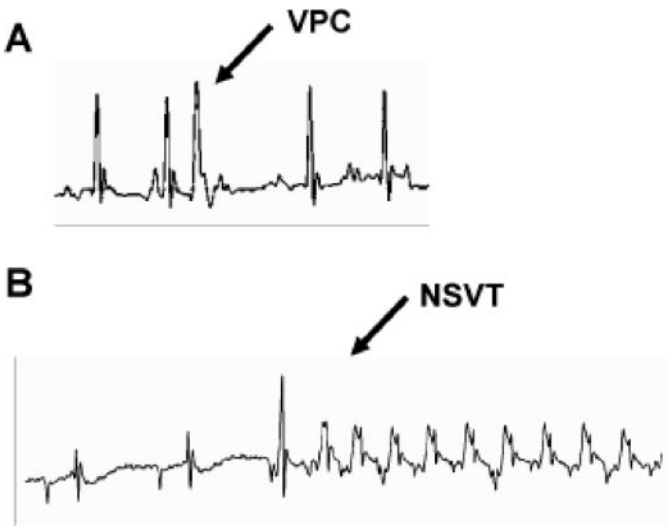

Fig. 6. Examples of arrhythmias recorded in *Kcnq1*$^{-/-}$ mice and isolated perfused mouse hearts. A, example of ventricular premature contraction (VPC, arrow) that was recorded by radiotelemetry. B, example of nonsustained ventricular tachycardia (NSVT) induced by nicotine in an isolated perfused *Kcnq1*$^{-/-}$ mouse heart. The RR value before nicotine exposure was 192 ms (heart rate ~313 bpm). The RR values during this episode of ventricular tachycardia were ~60 ms, corresponding to a heart rate of nearly 1000 bpm. The arrhythmia terminated spontaneously after approximately 1-min.

Discussion

Isolated Heart Experiments. In the present study, we showed that hearts lacking the ability to express Kcnq1 exhibited significantly longer JT, QT, and QTc intervals than their wild-type counterparts at peak nicotine-induced heart rates. These data were derived from ECG recordings of isolated mouse hearts perfused using a method originally described by Langendorff (1895) more than 100 years ago. Using modern ECG amplifiers, electrodes, and data acquisition systems, we were able to reliably record stable ECG signals using this approach. In previous studies, we have extensively used this strategy to measure QT intervals and various other ECG parameters from isolated Langendorff-perfused rabbit hearts (Ebert et al., 1998; Liu et al., 1998, 1999, 2003; Drici et al., 1999). Unlike the rabbit heart, however, the mouse heart does not typically generate a well defined T-wave under baseline perfusion conditions. Thus, it is more challenging to accurately determine the precise end of the T-wave in the mouse heart. To overcome this challenge, we averaged the mouse ECG signals over 30-s intervals (~150 beats at baseline), which minimized beat-to-beat variability. As we have shown previously (Casimiro et al., 2001) and confirm in the present study, this strategy produces relatively "clean" ECG signals and permits a reasonable estimation of the QT interval in the mouse heart. Furthermore, our data are consistent with action potential duration measurements from conventional and monophasic action potential electrode recordings in similar isolated perfused mouse heart preparations (Knollmann et al., 2001). Thus, despite the challenges associated with QT measurements in the mouse, we have great confidence in the accuracy of the data presented in this study.

The ECG responses were not specific to nicotine since epinephrine and isoproterenol produced similar effects when added to the perfusion buffer following washout of nicotine. Epinephrine is an endogenous stress hormone that activates both α- and β-adrenergic receptors, whereas isoproterenol is a β-selective agonist. Thus, our results show that regardless of whether sympathetic stimulation was provided by nicotine-induced release of norepinephrine from sympathetic nerve terminals/intrinsic cardiac adrenergic cells or by direct stimulation of cardiac muscle via adrenergic receptor agonists, a significantly greater QTc increase was observed in *Kcnq1*$^{-/-}$ hearts compared with *Kcnq1*$^{+/+}$ hearts, thereby suggesting that Kcnq1 expression is an important modulator of repolarization in the presence of catecholamines. This idea is further supported by recently published data which showed that cardiac-specific overexpression of KCNQ1-KCNE1 in a novel transgenic mouse model led to "enhanced shortening" of action potential duration (APD_{50}) following challenge with isoproterenol (Tracy et al., 2003).

Since long QT phenotypes were observed with nicotine, epinephrine, and isoproterenol in isolated perfused *Kcnq1*$^{-/-}$ mouse hearts in addition to intact *Kcnq1*$^{-/-}$ mice following injection/handling stress, the simplest explanation for these results is that cardiac Kcnq1 expression is important for efficient repolarization during sympathetic stimulation in the mouse. This point is clearly illustrated by the lack of QT adaptation to nicotine-induced increases in heart rate in Kcnq1-deficient mouse hearts. In *Kcnq1*$^{+/+}$ hearts, the QT interval generally became shorter as the heart rate increased. Thus, the lack of such an adaptation in *Kcnq1*$^{-/-}$ hearts suggests that Kcnq1 is an important downstream mediator of stress responses in the heart.

One curious finding observed in the present study was the lack of the initial slowing of heart rate in *Kcnq1*$^{-/-}$ hearts compared with *Kcnq1*$^{+/+}$ hearts immediately following exposure to nicotine. In isolated rabbit and rat heart preparations, this response is relatively brief and is thought to be a parasympathetic effect of nicotine because it can be blocked by pretreatment with atropine (Kottegoda, 1953; Ji et al., 2002). It can also be distinguished from the sympathetic response by pretreatment with α-bungarotoxin, an α7-nicotinic acetylcholine receptor antagonist (Ji et al., 2002). Although we did not examine nicotinic receptor subtypes in the present study, the overall heart rate responses to nicotine in the isolated perfused wild-type mouse hearts were qualitatively similar to those observed in the isolated perfused rat heart (Ji et al., 2002). The lack of this initial rate response to nicotine in *Kcnq1*$^{-/-}$ mouse hearts did not alter the subsequent increase in heart rate, but the significance of this finding clearly requires further investigation.

In Vivo ECG Analysis. Using surgically implanted ECG electrode transmitters and radiotelemetry monitoring devices, we were able to record ECG signals in freely moving, nonsedated mice. Analysis of QTc before and after injection stress demonstrated a small but significant increase (11 ± 3%) in *Kcnq1*$^{-/-}$ mice relative to *Kcnq1*$^{+/+}$ mice (−1 ± 1%). These results generally reflect our findings with the isolated perfused mouse hearts, further supporting the idea that Kcnq1 may be important repolarization during sympathetic stimulation.

A great advantage of the radiotelemetry approach is the ability to record ECGs without the use of restraints, sedatives, or anesthetics. Such interventions may influence ECG data and could account for some of the discrepancies observed between our previous data (Casimiro et al., 2001) and

those reported by Lee et al. (2000). For example, we reported that targeted disruption of the *Kcnq1* gene in mice produced extended QTc intervals and altered T-wave morphologies on ECG recordings from diazepam-sedated mice (Casimiro et al., 2001), a finding which is consistent with those reported for a dominant-negative *KCNQ1* cardiac-specific transgenic mouse model (Demolombe et al., 2001). Working independently, Lee et al. (2000) produced another mouse model that disrupted the *Kcnq1* gene, although they did not observe any ECG differences between $Kcnq1^{+/+}$ and $Kcnq1^{-/-}$ mice that had been anesthetized with metofane. In light of the present radiotelemetry results demonstrating that stress provokes a long QT phenotype in conscious mice, a plausible explanation for the apparent discrepancy between the previous studies is that since our mice were sedated (diazepam), they may have experienced some level of "stress" (e.g., handling, injection) that could have contributed to the observed ECG phenotype (Casimiro et al., 2001). In contrast, the mice from the Lee et al. (2000) study were anesthetized with metofane and were, therefore, presumably less prone to sympathetic stimulation. In addition, metofane clearly affects some sodium and potassium currents (Elliott et al., 1992), further complicating interpretation of ECG data obtained in animals anesthetized with this compound. These complications are eliminated by the use of radiotelemetry.

Relation to Human Studies. It is well established that mutations in the human *KCNQ1* gene can lead to congenital long QT syndrome (Yang et al., 1997; Schwartz et al., 2001), and it is generally believed that these mutations reduce or eliminate I_{Ks}. Due to the difficulty of performing detailed cellular electrophysiological assessments in human ventricular myocytes, there are relatively few published reports evaluating I_{Ks} in these cells (Li et al., 1996; Iost et al., 1998). Furthermore, these reports disagree as to the relative importance of I_{Ks} in the human heart (Veldkamp, 1998). In adult mouse and rat hearts, I_{Ks} is scarce due to the developmental down-regulation of *Kcne1* (formerly, *isK* or *minK*) (Honore et al., 1991; Drici et al., 1998). Given the potential for Kcnq1 to function as a homomeric channel and/or "partner" with multiple members of the Kcne(1–5) family of modulators (Barhanin et al., 1996; Sanguinetti et al., 1996; Yang et al., 1997; Schroeder et al., 2000; Tinel et al., 2000; Abbott et al., 2001; Angelo et al., 2002; Grunnet et al., 2002; Mazhari et al., 2002), it is possible that Kcnq1 contributes to K^+ currents other than I_{Ks} in mouse (and human) myocardial cells.

People with the LQT1 form of long QT syndrome are at high risk for developing TdP arrhythmias; however, we observed no such arrhythmias in our model under any of the conditions employed. Furthermore, it is unclear if the occasional VPCs and NSVTs that we recorded in vivo and in vitro were directly related to the absence of Kcnq1 (because of the low incidence of their occurrence and the relatively small number of mice analyzed). The generation of arrhythmias in the context of delayed repolarization is a complex process that is thought to be triggered by early after depolarizations and/or dispersion of repolarization. Given the significant cardiac electrophysiological differences that are known to exist between mice and humans, it appears that $Kcnq1^{-/-}$ mice may be relatively resistant to developing TdP arrhythmias even though our results clearly show that QT-lengthening reliably occurred in Kcnq1-deficient mouse hearts during sympathetic stimulation. Conceivably, differences in other currents (e.g., the predominance of the transient outward current, I_{to}, in the adult mouse heart) (Wang and Duff, 1997) and/or the underlying anatomical substrates that facilitate TdP arrhythmias in humans could account for the lack of such arrhythmias in $Kcnq1^{-/-}$ mouse hearts.

Despite this limitation of the mouse model, our data lend support to the hypothesis that sympathetic stimulation represents an additional risk factor for long QT patients. The acquired form of long QT syndrome is much more prevalent than the congenital form (Faber et al., 1994), and it has many contributing factors such as electrolyte imbalances, metabolic disorders, heart disease, female gender, and most notably, drugs that block delayed rectifier potassium currents (primarily I_{Kr}) in ventricular myocytes (Roden and Spooner, 1999). The primary K-channel subunit responsible for I_{Kr} is encoded by the human *ether-a-go-go*-related gene (*HERG*), and mutations in this gene comprise a second form of congenital long QT syndrome, LQT2 (Sanguinetti et al., 1995). Conceivably, if HERG channel function is impaired by mutations or drugs, then the remaining outward K channels, such as those encoded by *KCNQ1* and other genes, may then play a more prominent role in mediating cardiac repolarization. Interestingly, LQT2 patients also appear to be at high risk for developing arrhythmias during periods of acute stress (Schwartz et al., 2001). Indeed, β-blockers provide effective therapy for LQT2 and LQT1 patients, although efficacy is greatest for LQT1 (Schwartz et al., 2001). Nevertheless, since the acquired form of long QT syndrome often involves drug blockade of the HERG channel that has been linked to LQT2, sympathetic influences may also play a prominent role in acquired long QT syndrome.

The data reported here suggest that the murine $Kcnq1^{-/-}$ genotype faithfully reproduces a long QT phenotype (albeit without the associated TdP arrhythmias) during sympathetic stimulation and may, therefore, provide useful insight regarding the biological function of cardiac *Kcnq1* expression. It is of potential interest to note that in LQT1 patients β-blockers appear to provide effective therapy, even causing shortening of the QTc interval in some patients (Conrath et al., 2002). This implies that the LQT1 phenotype is driven, at least in part, by sympathetic stimulation in humans, a finding that also appears to hold true in the mouse.

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Address correspondence to: Dr. Steven N. Ebert, Department of Pharmacology, Georgetown University Medical Center, 3900 Reservoir Rd., NW, Washington, DC 20057. E-mail: eberts@georgetown.edu